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↓ 14K 10'
↓ 1ml 70% EtOH
↓ 2'
↓ air dry
pellets

The Amount of DNA of #2 and #4 products is about 1.8 ug.
Amount of DNA of #7, #8, #5 and #4 products is about 28 ug.
90 ul and 140 ul H2O to make their final concentration at about 0.2 ug/ul.
Store them at −20°C.

4. PCR OS 6-19-98

Now I am doing PCR analysis since the PCR Test worked fine.

DNA	Primers	Product	bps	Comments
pBR322, PstI	3+5	O1	1535	
"	1+5	O2	813	
pUC19, RI	1+5	O3	813	
pACYC177, BHI	4+5	O4	1011	
"	1+5	O5	726	
pBR322, PstI	2+5	O6	1057	
pACYC177, BHI	1+6	O7	699	
"	4+6	O8	979	
pACYC184, BHI	1+6	O9	694	
pBR322, PvuII	7+11	S1	1130	
pUC19, RI	7+11	S2	1130	
pACYC177, BHI	7+11	S3	1130	
"	8+12	S4	1219	
pBR322, PvuII	9+13	S5	1552	
pACYC184, BHI	10+14	S6	1104	

DNA are from page 6. They are diluted to 8 ng/ul and 1 ul was used for each reaction.
Primers are from page 5. The estimated concentration is about 8 ng/ul. 2 ul of each primer was used.
Dilute the DNA into appropriate concentration.
Making master mix as on page 6 except the number of reactions should be 16 instead of 12. One extra is for negative

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sed & Understood by me,	Date	Invented by	Date
	6/18/98	Recorded by	6-19-98

Page No. & control.

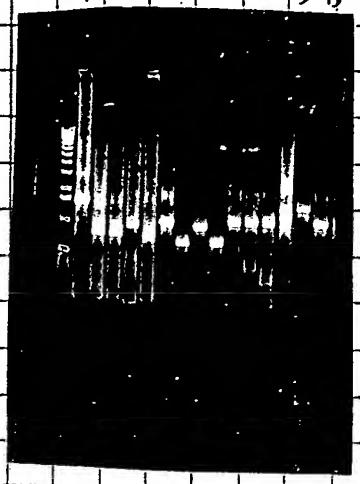
The PCR condition is same as on page 7.

100 ul of each PCR product

↓ take out 3ul

6/24/98 ↓ run on 0.8% agarose TBE

← ↓ take a picture



All the reactions worked. However, some worked better than others. #1 to 5 and 10 to 14 seems have many non-specific products. By increasing the template concentration, specific product may be increased and non-specific products may be decreased.

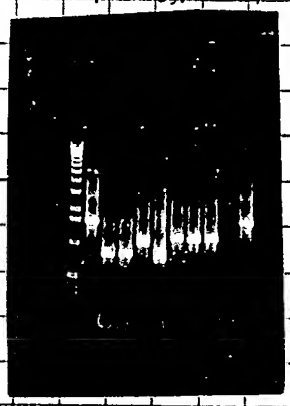
6-20-98 → Repeat reactions #1 to 5 and 10 to 14 by increasing the template 10X (use 10ng)

100 ul of each PCR product

↓ take out 3ul

↓ ran on 0.8% agarose TBE

6/24/98 ← ↓ take a picture



All the reactions appear to be better except #13. I probably made mistake when adding template or primers for #13. Keep the PCR products at -20°C

5. PCR SI

6-21-98

	DNA	Amount	Primers	P	bps	Comments
a	pACYC177 BH1	1 ng	8+12	S4	1219	
	"	10 ng	"	"	"	
c	pACYC177 P+E	1 ng	"	"	"	
d	"	10 ng	"	"	"	
	pGEX -3X	1 ng	15+17	I	1198	

The primers are from page 5. 2ul of each primer was used. The PCR condition is same as on page 7.

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Used & Understood by me,
STEVEN F. GESSERT

Date 6/24/98

Invented by Chuanli

Recorded by Chuanli

Date 6-20-98, 6.21.98

Page No. 92 ② By comparing the yields of the minipreps between page 11 and 92, it is clear that the copy number of plasmids is determined by the selection marker it contains. In OS4 minipreps, the A based plasmids give very low yields except #15.

③ ~~The~~ SmaI has two sites on OS4 constructs. Therefore two bands generated after SmaI digestion. However the size of the DNA appear be different even from the colonies picked from same plate. This observation may be artifacts of electrophoresis, but I need pay attention on this observation in further analysis.

22. OS Médiprep Test 1 9.8.98

Use the residual O/N cultures from 4 ml innoculation (These residual ones are kept at 4°C) to seed 5 ml LB with appropriate antibiotic. prep number, page and O/N culture number are indicated in the following table.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
84	84		[illegible] 87	87	85	87	85		91	[illegible] 87	91		92		
2	28	[illegible]	[illegible]	18	22	21	25	[illegible] 19	[illegible] 8	[illegible] 13	16	2	[illegible] 8	10	15
	Amp					Tet				chl			Kan		

The above table is messy. I will re-prepare the table below:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Amp					Tet				chl			Kan		
84	84	[illegible]	87	87	85	87	85	91		87	91		92		
2	28	16	4	18	22	21	25	19	8	13	16	2	8	10	15

5 ml innoculated LB w/ appropriate antibiotic

↓ 37°C w/ shacking at 300 RPM for 3 h,

1 to 4, 5, 7, 13 and 15 grows fast.
6, 8, 9 to 12, 14 and 16 grow slowly.

↓ 37°C for another 3 h w/ shaking

9 to 12, 14 and 16 still do not grow well

↓ 37°C w/ shacking O/N. ~13 hs

9.9.98 innoculate all 5 ml into 50 ml LB w/ antibiotic

↓ 37°C for 1 h w/ shacking

Test OD590
#1 : 0.435 #12 : 0.278

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Witnessed & Understood by me, [signature] Date 9-11-98

Invented by [signature] Date 9.8.98

Recorded by [signature]

↓ 37°C for another 1h w/ shaking.

Test OD575

#1 : 0.718 #12 : 0.283
#8 : 0.876 #16 : 0.257

The final concentration of chl 5×35/50 = 10 ug/ml

↓ add 15 ul 35mg/ml chl to 1-8 and 13-16

↓ 37°C w/ shaking for 4 hs

#1 : 1.260 #12 : 1.192
#8 : 0.992 #16 : 0.337

↓ 3.2K for 5' (actually 10' should be enough)

pellets (#16 has smallest pellet)

also has a small pellet and 11 have biggest pellets. are most viscous after suspend the cells

↓ 0.5 ml LETT

↓ completely resuspend cells by pipetting

↓ transfer to eppendorf tubes

↓ boil for ~~90°~~ → 120°

↓ → 14K 10'

after 10' spinning, #1-5, 7, 8, 13-15 did not pellet, boil in for another 3' and repeat spinning.

*1 → *2 → *4

↓ transfer supernatants to new tubes

supernatants ×3

after second spin, they form a pellet. Freeze them in ice/EtoH bath, thaw them, spin for another 10' (#1-5, 7, 14, 15 are treated dry ice/EtoH bath)

↓ 1V ϕ-Chl ext.

↓ 2V EtoH

↓ 5' 14K

pellets

adjust the supernatants to the same volume by LETT

↓ 70% EtoH Wash

pellets

↓

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ssed & Understood by me,	Date	Invented by	Date
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om Page No. 94 *4 The lysed cell pellets are different in size. The approximate pellet size (CAPS) are listed in the following table:

sp#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PS	400	400	300	250	400	100	250	200	200	200	150	120	100	250	250

The approximate pellet sizes (CAPS) are in microliters.

pellets after 70% EtOH wash are different in size. Their relative size are listed in the following table:

sp#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ize	L	L	L	L	L	~~L~~ S	L	M	S	S	S	VS	L	L	L

↓ air dry the pellets O/N.

↓ re-suspend in 200ul TE w/RNase A 9.10.

After O/N air dry, the pellets are difficult to be desolved especially the larger pellets. When they are finally desolved (take about 2hs w/ vortexing), they form heavy foams while vortexing.

Add another 200ul TE w/RNase A to large pellet tubes namely #1-5, 7, 13-15.

It is amazing that all the pellets seem desolve completely after these efforts.

SmaI digestion:

master mix for each RXN	16RXNs
H2O 6.9 ul	110.4
10X Buffer 1 ul	16
SmaI 0.1 ul	1.6
8 ul/RXN	128 ul totally.

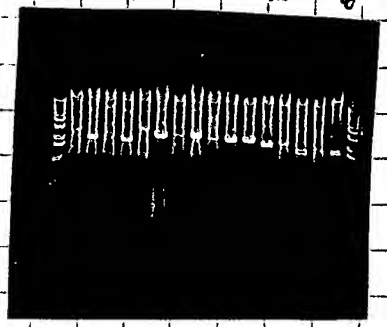
take out 2ul from each sample

↓ add 8ul master mix

↓ R.T for 1h

↓ run on 0.8% agarose TBE

↓ take a picture. (416, 1/2 sec)



Result analysis:

① Genomic DNA contamination is serio
possible solutions ⓐ Decrease the boiling t
ⓑ Do not use pepitt to resuspend the cell
ⓒ Decrease the Triton-X100 Concentration.
ⓓ Most of the preps have enough DNA for future usage. However 3, 7 and 15 appe to have very little DNA (0.025 per band).

possible solutions ⓐ use higher concentration of chloramphenicol on

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tnessed & Understood by me,	Date	Invented by	Date
	9-11-98	Recorded by	9-9-10.98